

Remarks

Applicants respectfully request that this Preliminary Amendment and Submission of Sequence Listing be entered by the Examiner. This amendment amends the specification, cancels claims 1-38, 40, and 43-47, rewrites claims 39, 41 and 42, and inserts new claims 48-75. No new matter has been added.

The specification has been amended to direct the entry of the Sequence Listing after the claims of the above identified application and to provide the SEQ ID NO's next to the specific sequence. In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same. Also submitted herewith is a Request to Open New Disk File for the present application, indicating that the Substitute Sequence Listing disk submitted on April 5, 2001, in the parent Appl. No. 09/220,077, filed December 23, 1998, contains the identical sequence information as that in the Sequence Listing submitted herewith.

Claims 39, 41 and 42 have been rewritten to independent form and include the subject matter of original claim 1. Further, claims 39, 41 and 42 have been amended to replace the recitation "the mutein" with --one or more muteins--. Support for this amendment can be found at page 18, lines 1-2 and 10. Furthermore, claims 39, 41 and 42 have been amended to recite that the numbering of amino acids is based on SEQ ID NO:1. Support for this can be found at page 7, lines 7-8. It is respectfully submitted that Applicants intention is not to limit amended claims 39, 41 and 42 to the 157 amino acid

form of basic human fibroblast growth factor, but to make them more clear. Claim 42 has also been amended to include the subject matter of canceled claims 43-47.

Support for the new claims 48-75 can be found in the specification and original claims. Claims directed to the mutein recited in new claim 61 were allowed during the prosecution of the parent application No. 09/220,077.


Upon entry of the foregoing amendment, claims 39, 41, 42, and 48-75 are pending in the application, with claims 39, 41 and 42 being the independent claims. As such, no new matter has been introduced into the captioned application.

Applicants respectfully request that this Preliminary Amendment under 37 C.F.R. § 1.111 be entered by the Examiner. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this application is respectfully requested.

Respectfully submitted,

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SKGF Rev. 2/13/01

Version with markings to show changes made

In the Specification:

1st full paragraph on page 1:

This is a division of U.S. Patent Application Serial No. 09/220,077, filed December 23, 1998, which [application] claims the benefit, under 35 U.S.C. § 119(e), of the earlier filing date of [provisional application] U.S. Provisional Application, Appl. No. 60/068,667, filed on December 23, 1997[, which is herein incorporated by reference]. The entirety of each of these applications is incorporated by reference herein.

3rd full paragraph on page 6:

Figure 1. This figure presents the DNA (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of the 157 amino acid form of bFGF used in this invention. The N-terminal, initiating methionine is processed by *E. coli*, and the purified bFGFs, therefore, lack the first amino acid shown in the figure. The nucleic acid sequence reported in this figure is representative of the wild-type bFGF DNA sequence following modifications of the gene purchased from R&D Systems. The DNA modifications were performed to incorporate restriction sites for subcloning and cassette mutagenesis; in all cases, the original amino acid sequence remained unaltered. The boxed and numbered amino acids identify those subjected to site-directed mutagenesis.

The paragraph beginning on page 12, line 29:

The mutein of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the mutein of the present invention. The marker sequence may be a hexa-histidine tag or the T7 peptide (amino acid sequence: Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly (SEQ ID NO:4)) supplied by a vector to provide for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al.*, *Cell* 37:767 (1984)). Other marker sequences well known to those skilled in the art may be used for similar purposes.

The paragraph beginning on page 28, line 18:

All other mutations reported in Table 1 were constructed using cassette mutagenesis. The L137A mutation was made by inserting complementary oligonucleotides into the AflIII and ApaI sites in which the CTT codon for leucine was replaced with the GCT codon for alanine (Figure 1). A HindIII site (not shown) was removed for convenient screening of positives. The DNA sequence for the above mentioned mutations differs somewhat from that which appears in Figure 1 although the amino acid sequence remains identical except for the introduced site-directed mutations. The DNA sequence for the E89A, E89Y, D101A and L137A mutants differs from that reported in Figure 1 only between the KpnI and ApaI restriction enzyme sites. Except for the introduced mutation (boxed codon in Figure 1) the following DNA sequence between the KpnI and ApaI sites is representative for the E89A, E89Y, D101A and L137A mutants:

CTGGCTATGAAGGAAGATGGAAGATTACTGGCTTCTAAATGTGTTACGGATG
AGTGTTCCTTTTTTGAACGATTGGAATCTAATAACTACAATACTTACCGCTCG
AGAAAATACACCAGTTGGTATGTGGCACTTAAGCGTACCGGTCAGTACAAGC
TTGGTTCTAAAACGGGCC (SEQ ID NO:3). The E89A and D101A double mutation reported in Table I was constructed by inserting complementary oligonucleotides into the KpnI and XhoI sites (Figure 1). The E89A, D101A, L137A triple mutant was constructed by combining the E89A, D101A double mutant with the single L137A mutant using the restriction enzyme sites engineered into the DNA sequence (Figure 1). All mutations were confirmed by DNA sequencing of plasmid DNA. Cassette mutagenesis was performed using the bFGF expression construct (see below) such that no further subcloning was required.

In the Claims:

Claims 1-38, 40, and 43-47 have been canceled.

New claims 48-75 have been inserted.

Claims 39, 41 and 42 have been amended as follows:

- 39.(Once amended) A method of stimulating cell division which comprises:
[(a)] (A) contacting cells with an effective amount of [the] one or more
[mutein] muteins of [claim 1] a human basic fibroblast growth
factor, or biologically active peptides thereof in vitro, wherein said
one or more muteins comprise the substitution of a neutral and/or
hydrophobic amino acid for one or more of the following:
(a) Glutamate 89; or
(b) Aspartate 101; or
(c) Leucine 137;
wherein the numbering of amino acids is based on SEQ ID NO:1;
or

[(b)] (B) contacting cells with an effective amount of [the] said one or more [mutein] muteins [of claim 1], or biologically active peptides thereof *in vivo*.

41. (Once amended) A method of healing a wound comprising contacting said wound with an effective amount of [the] one or more [mutein] muteins of [claim 1] a human basic fibroblast growth factor, or biologically active peptides thereof, wherein said one or more muteins comprise the substitution of a neutral and/or hydrophobic amino acid for one or more of the following:

- (a) Glutamate 89; or
- (b) Aspartate 101; or
- (c) Leucine 137;

wherein the numbering of amino acids is based on SEQ ID NO:1.

42. (Once amended) A method of treating ischemia, peripheral vascular disease, a neural injury, a gastric ulcer, a duodenal ulcer, or heart disease comprising contacting cells with an effective amount [the] one or more [mutein] muteins of [claim 1] a human basic fibroblast growth factor, or biologically active peptides thereof, wherein said one or more muteins comprise the substitution of a neutral and/or hydrophobic amino acid for one or more of the following:

- (a) Glutamate 89; or
- (b) Aspartate 101; or
- (c) Leucine 137;

wherein the numbering of amino acids is based on SEQ ID NO:1.